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13. ABSTRACT (Maximum 200 Words)

Prospective studies indicate that as body weight and/or energy intake increase so does the risk for prostate cancer. A protective effect of energy restriction on development of spontaneous prostate tumors in Lobund-Wistar rats and tumors developing from transplanted prostate tumor tissue or cells in mice and rats have been published, but a mechanism of action has not been identified. Recent introduction of the TRAMP (transgenic adenocarcinoma mouse prostate) mouse provides a model that shares characteristics with human prostate cancer. Here, TRAMP mice are being used to evaluate their response to chronic and intermittent calorie restriction. The insulin like growth factor (IGF) axis is being investigated to determine if it is involved in this protective process. TRAMP mice are enrolled in ad libitum-fed, intermittent-restricted and chronic restricted groups in both longitudinal and cross sectional study to determine prostate cancer incidence, latency and metastasis rate. A 25% reduction in caloric intake is being utilized. Initial findings indicate that more of the intermittent-restricted mice are surviving until the designated end point of the study. Evaluation of histopathology is underway and we are attempting to identify a metabolic pathway to target for prevention and/ treatment strategies.

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Table of Contents

INTRODUCTION:

A number of prospective epidemiological studies indicate that as body weight and/or energy intake increase so does the risk for prostate cancer. In rodent studies chronic calorie restriction is associated with extended life expectancy and decreased incidence of many malignancies. Due to a lack of suitable animal models of prostate cancer, only a few studies have addressed issues of nutrition intervention in the progress of this disease. However, results of these studies support a protective effect of energy restriction on spontaneous prostate tumor development in Lobund-Wistar rats [1;2] and on transplanted tumor/cell prostate tumor growth in mice and rats [3], although a mechanism of action has not been identified. There are limitations to the application of these models to the human disease process. Recent introduction of the TRAMP (transgenic adenocarcinoma mouse prostate) mouse provides a model that shares many characteristics with human prostate cancer [4;5], but their use in nutritional studies has been limited. We are using TRAMP mice to evaluate their response to chronic calorie restriction, as well as to intermittent caloric restriction/refeeding. These studies are based on our recent report that these two interventions resulted in decreased incidence and extended latency of oncogene-induced mammary tumors in MMTV-TGF-α female mice [6]. Furthermore, we found that the intermittent caloric restriction/refeeding regimen was more protective that chronic restriction. TRAMP mice are being followed to determine their response to these interventions with respect to age of prostate cancer detection and metastases rates. Serum and tissue samples has been obtained to determine the role of the insulin-like growth factor (IGF) axis in the protective action of caloric restriction.

BODY:
Progress in relation to Revised Statement of Work 2/13/03 (attached)

TASK 1 & 2. Establish breeding colony & set up genotyping assay.
MONTH 0-3. Order 6 male TRAMP mice (maximum number that could be ordered at one time) and 25 nontransgenic female mice for breeding. Set up breeding. Set up genotyping assay and genotype mice produced. Rebreed mice to expand breeding colony.
Initially as reported in the first progress report we had some problems in establishing the breeding program. This has been addressed although overall breeding has been less productive than originally anticipated. ***Now completed with respect to breeding regimens and genotyping.***

TASK 3. Breed mice for EXPERIMENT 1A- LONGITUDINAL STUDY.
MONTHS 4-6. Breed mice to produce one third to one half of mice needed for this study. If one estimates 8 pups per litter, 1 out of 4 pups will be TRAMP males = two TRAMP males per litter. We will need a total of 160 TRAMP males = 80 litters. Genotype offspring. Assign mice to experimental groups. Set up immunohistochemistry assays.
Progress on TASK 3. *Response in first report.* During this phase we concentrated on enrolling mice in the Longitudinal Study as we were running behind schedule due to the problem cited above. Due to the focus on genotyping and taking care of mice no other assays were set up.
Update on Task 3. See Task 4.

TASK 4. Complete enrollment of mice for EXPERIMENT 1A and 1B SERIAL STUDY.
MONTHS 7-12. Continue breeding to compete EXPERIMENT 1A. Three to four rounds of breeding will probably be needed to supply enough mice. Genotype mice as they are produced. Assign mice to experimental groups. Once longitudinal study is complete begin assigning mice to serial study for EXPERIMENT 1B.
Progress on TASK 4. *Response to first report.* Almost all mice are entered in the Longitudinal study and mice are being assigned to a Cross-sectional group which will be euthanized in the summer (2004) when we have a summer intern involved in the project .
Update on Task 4. All mice have been enrolled in the Longitudinal study (Experiment 1A) (Table 1). In addition as shown in Table 2 all mice except four have been enrolled in the Cross Sectional study. Thus the breeding took quite a bit longer than originally anticipated.

Table 1. Mice Enrolled in Experiment 1A- Longitudinal Study (4/8/05)

	Enrolled TRAMP MICE [enrolled nonTRAMP mice for age-matched comparison]	TRAMP MICE TO BE ENROLLED [enrolled non-TRAMP mice for age-matched comparison]
AD LIBITUM-FED	40 [15]	0 [0]
INTERMITTENT RESTRICTED/REFED	101* [33]	0[0]
PAIR-FED	80 [20]	0[0]
TOTALS	221 [68]	0[0]

*additional TRAMP mice were enrolled to provide more tumor samples at the 50 week end point

Table 2. Mice Enrolled in Experiment 1B- Cross Sectional study (4/8/05)

	Enrolled TRAMP MICE [enrolled nonTRAMP mice for age-matched comparison]	TRAMP MICE TO BE ENROLLED [enrolled non-TRAMP mice for age-matched comparison]
AD LIBITUM-FED	49 [37]	0[0]
INTERMITTENT RESTRICTED/REFED	51 [34]	0[0]
PAIR-FED	48 [33]	4[0]
TOTALS	148 [104]	4[0]

TASK 5. Follow mice in EXPERIMENT 1A and 1B.

MONTHS 6-21. Monitor food intake, body weight and prostate tumor development in TRAMP mice. When age, tumor size and/or animal condition dictates euthanize mice and perform autopsies. Euthanize nontransgenic age-matched mice to correspond to those TRAMP mice with tumors. Euthanize mice that reach terminal ages of 48 or 50 wk of age. Record results and when study complete do statistical analyses of results.

Progress on TASK 5.

Number of mice enrolled. Presently 276 mice are being followed. Due to the fact that we now must house the mice individually in shoe-box type caging rather than hanging wire-mesh cages as originally planned there are some limitations as to the number of mice that can be housed at a given time. Also the initiation of per diem animal costs by the Hormel Institute after this project was funded has resulted in a financial cost to the grant that was not originally planned.

Food Intake. Food intakes for the mice during each of the four week cycles of the experiment are shown in Table 3. By prevention of overeating during the refeeding period we are maintaining an overall degree of restriction of ~25% for the intermittent-restricted and chronic-restricted (pair-fed) groups. This should make interpretation of the results more straight forward as we found previously with female mice that some of them overate relative to the ad libitum fed mice during the refeeding stages resulting in overall caloric restriction being in the range of 10-20% although still highly protective [7] (Cleary et al manuscript in preparation).

Table 3. Summary of food intake data (mean grams/day ± sd) for TRAMP mouse study (4/8/05)

	Ad libitum-fed	Intermittent-Restricted	Chronic-Restricted
Cycle 1	5.63 ± 0.79 (n=140)	4.2 (n=208)	4.2 (n=180)
Cycle 2	5.33 ± 0.69 (n=140)	4.0 (n=200)	4.0 (n=180)
Cycle 3	5.12 ± 0.46 (n=118)	3.8 (n=172)	3.5 (n=156)
Cycle 4	4.91 ± 0.71 (n=99)	3.7 (n=165)	3.7 (n=147)
Cycle 5	4.65 ± 0.72 (n=78)	3.5 (n=157)	3.5 (n=140)
Cycle 6	4.46 ± 0.70 (n=54)	3.3 (n=132)	3.3 (n=98)
Cycle 7	4.41 ± 0.51 (n=39)	3.3 (n=117)	3.3 (n=69)
Cycle 8	4.29 ± 0.41 (n=29)	3.2 (n=85)	3.2 (n=61)
Cycle 9	4.25 ± 0.50 (n=27)	3.2 (n=79)	3.2 (n=51)
Cycle 10	4.19 ± 0.39 (n=17)	3.1 (n=72)	3.1 (n=37)
Cycle 11	4.13 ± 0.76 (n=11)	3.1 (n=23)	3.1 (n=27)

Number in parentheses = number of mice. Table includes results from both longitudinal and cross-sectional study.

Body weight curves. The body weight curves for the mice as of 4/8/05 are shown in Figure 1. Since the study is ongoing statistics have not been done. In contrast to our earlier study using female TGF-α mice the intermittent restricted mice are not regaining weight to reach the body weight attained for the ad libitum-fed mice. However, as indicated above in this protocol we are restraining their food intake during the refeeding periods so that they do not "overshoot" the intake of the ad libitum fed mice. For clarity of presentation the error bars for the two restricted groups have not been included.

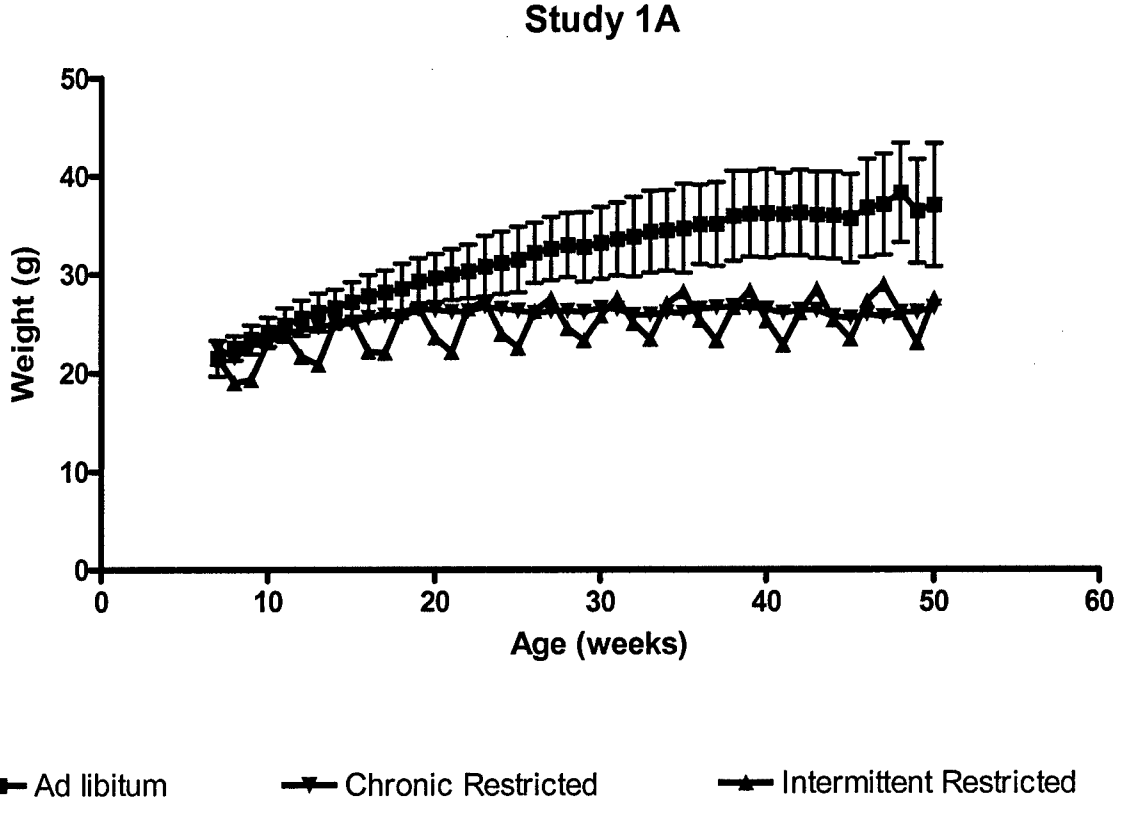

Figure 1: Body Weight curves for male mice in longitudinal study. (Ad libitum, n=11-55 depending upon age; Intermittent-Restricted, n=23-134 dependent upon age; Chronic-Restricted n=27-100 dependent upon age). No statistics done at this point in time. For clarity error bars not included for the two restricted groups.

Overall progress of Longitudinal Study- 1A:
At the present time 38 mice remain alive in this part of the study with scheduled euthanasia (48 or 50 weeks of age) May through mid-September. To date 251 mice have been sacrificed or died. A summary of some of the results obtained from these mice is presented in Table 4. TRAMP mice results are shown in bold with smaller groups of nonTRAMP mice included for comparison purposes.

Table 4. Summary of Results from Longditudinal TRAMP 1A Study. (mean value with sd).

	N	Tumor number	Tumor weight (g)	GU weight including tumors (g)	Age at palpation (weeks)	Age at euthanization (weeks)	# who lived to terminal

							age
Ad libitum TRAMP	**34**	**2.00 (2.84)**	**2.86 (2.74)**	**5.47 (2.13)**	**33.6 (8.41) min: 18 max: 50 N=31**	**37.4 (9.79) min: 16 max: 50**	**4**
Ad libitum non-TRAMP	12	0	0	1.28 (0.20)	n/a	45.8 (9.82)* min: 22 max: 50	10
Intermittent Restricted TRAMP	**79**	**1.52 (1.23)**	**2.77 (2.54)**	**4.15 (2.60)**	**36.6 (8.17) min:21 max:48 N=62**	**40.47 (9.20) min:21 max:50**	**29**
Intermittent Restricted non-TRAMP	32	0	0	0.84 (0.23)	n/a	47.9 (5.40) min: 27 max: 50	31
Chronic Restricted TRAMP	**65**	**1.57 (0.85)**	**3.14 (2.52)**	**4.10 (2.51)**	**34.0 (7.69) min: 21 max: 50 N=54**	**38.4 (9.71) min: 14 max: 50**	**16**
Chronic Restricted non-TRAMP	15	0	0	0.85 (0.20)	n/a	50 (0) min: 50 max: 50	15

None of the results presented are at this point are statistically significant with respect to comparisons among the three TRAMP groups (bold type).However, it is interesting to note that only 9% of the Ad libitum-fed TRAMP mice survived until 50 weeks of age while 37% of the Intermittent-Restricted mice lived to their terminal end point (48 or 50 weeks) did as did 25% of the Chronic-Restricted mice. This is the most noticeable difference to date. However there is a trend towards a protective effect of caloric restriction on the development of prostate cancer appears to be emerging as summarized in Table 5.

Table 5. Comparisons Among TRAMP Mouse Groups in Longitudinal Study.

	Ad libitum vs Intermittent-Restricted	Ad libitum vs Chronic-Restricted	Intermittent-Restricted vs Chronic-Restricted
Food intake	25% ↑	25% ↑	same
Tumor Number	32% ↑	27% ↑	same
Tumor Weight	same	9% ↓	12 % ↓
Age at Tumor Palpation	8% ↓	same	8% ↑
Age at Death	8% ↓	same	5% ↑
Survival until study termination	90% ↓	84% ↓	48 % ↑

We have recently received a large number of pathology results and we are documenting and organizing them in order to determine the extent of disease involvement and prostate cancer incidence and the extent of metastases. We will then start study of these tissues

Over all Progress on Cross sectional Study 1B:
In this aspect of the study 149 mice have been euthanized or have died and 103 remain. Mice are scheduled to be euthanized April through November. At this time the results have not been analyzed since a large number of mice remain to be euthanized at different ages.

ORIGINAL-TASK 6. Oncogene and tumor suppressor assays.
MONTHS 6-21. Order supplies and set up assays to perform p53, ErbB2 and possibly other growth factors for determination of gene expression and protein levels Complete setting up assays and analyze samples as they become available. (reviewers indicated not to do this)
REVISED-TASK 6. IGF-BP and IGF-I receptors. Since we are focusing on IGF metabolism any work relating to gene expression will concentrate on factors related to IGF-I action such as IGF-I, IGF-I receptors and IGF-BP's.
Progress on Task 6. The original postdoctoral fellow Xin Hu who was to work on this project left to accompany her husband to Penn. State Medical College prior to the beginning of the study. Despite having received over 100 applications for the position most of the remaining applicants did not have the appropriate background. The few that were qualified were not available by the time I contacted them. I therefore hired a well qualified individual at the technical level, Melissa Bonorden to get the study underway. She has been genotyping the mice and monitoring their food intake and health status. A second postdoctoral search was initiated and more successful. Dr. Olga Rogozina who has both an M.D. and Ph.D. join our research group May of 2004. She is working 50% effort on this project and will be doing molecular biology studies of the tissues as soon as we have gone through the pathology reports and determine what specific parts of the GU tract to use.

TASK 7. Restock breeding colony.
MONTHS 12-14. Evaluate breeding colony status and initiate breeding for EXPERIMENT 2-FASTING/REFEEDING study.
Progress on Task 7. The breeding colony has been continually restocked as we have been enrolling mice steadily over the course of the grant.

TASK 8. Enroll mice in FASTING/REFEEDING STUDY.
MONTHS 14-21. Breed mice, genotype offspring and enroll mice in FASTING/REFEEDING study. For this study 80-120 mice will be needed depended upon adding a PAIR-FED or a RESTRAINED group. We will have to follow the eating pattern of the FASTING/REFEEDING group for several months to determine if the additional group is needed.
Progress on Task 8. Due to the longer time frame to enroll the mice in the Intermittent Caloric Restriction studies, mice have not been enrolled in the Fasting/Refeeding study. Also as indicated above the Hormel Institute instituted Per Diem costs for animal maintenance and it was necessary to pay these costs which has resulted in less money available to carry out these studies. In addition, we do not feel it was prudent to start a new study until we have some idea of what are the results of the present study. If it is not successful it is unclear whether fasting/refeeding will provide a beneficial effect. We are presently waiting for results from pathology to assess what is happening and if possible we may institute either a shorter-term

fasting refeeding study or it critical periods are identified from the ongoing tissue and serum analyses a shorter term study focused on that would be undertaken.

TASK 9. Serum and tissue analyses and data analyses.
MONTHS 21-24 Complete tissue assays and when all animals are euthanized perform serum analyses and then complete data analyses of EXPERIMENT 1A and 1B.
Progress on Task 9. *Serum analyses will begin shortly with the completion of the longitiudinal study. Mice are still being maintained in both the Longitudinal and Cross-sectional studies so all samples can not be analyzed yet. We will focus our efforts in the last year of the project on serum and tissue analyses and statistical evaluation of the results.*

KEY RESEARCH ACCOMPLISHMENTS:

The major accomplishment is our completion of enrollment of the mice in the caloric restriction protocols and following their progress. The recent evaluation of results suggesting that intermittent caloric restriction may provide greater protection against prostate cancer than does chronic restriction is very exciting and we plan to aggressively pursue identifying tissue characteristics and pathways associated with this protective effect.

REPORTABLE OUTCOMES:

There are none yet. We hope will submit an abstract for presentation at the AACR 4th Annual Frontiers in Cancer Prevention Conference scheduled for October, 2005.

CONCLUSIONS:

Intermittent caloric restriction regimens may provide a useful tool for prostate cancer prevention.

Reference List

1. Pollard,M., Luckert,P.H., and Snyder,D.L. (1989) Prevention of prostate cancer and liver tumors in L-W rats by moderate dietary restriction. *Cancer*, **64**, 686-690.

2. Snyder,D.L., Pollard,M., Wostmann,B.S., and Luckert,P. (1990) Life-span, morphology, and pathology of diet-restricted germ-free and conventional Lobund-Wistar rats. *J.Gerontol.*, **45**, B52-B58.

3. Mukherjee,P., Sotnikov,A.V., Mangian,H.J., Zhou,J.-R., Visek,W.J., and Clinton,S.K. (1999) Energy intake and prostate tumor growth, angiogenesis, and vascular endothelial growth factor expression. *J.Natl.Cancer Inst.*, **91**, 512-523.

4. Greenberg,N.M., DeMayo,F., Finegold,M.J., Medina,D., Tilley,W.D., Aspinall,J.O., Cunha,G.R., Donjacour,A.A., Matusik,R.J., and Rosen,J.M. (1995) Prostate cancer in a transgenic mouse. *Proc.Natl.Acad.Sci.USA*, **92**, 3439-3443.

5. Gingrich,J.R., Barrios,R.J., Morton,R.A., Boyce,B.F., DeMayo,F.J., Finegold,M.J., Angelopoulou,R., Rosen,J.M., and Greenberg,N.M. (1996) Metastatic prostate cancer in a transgenic mouse. *Cancer Res.*, **56**, 4096-4102.

6. Cleary,M.P., Jacobson,M.K., Phillips,F.C., Getzin,S.C., Grande,J.P., and Maihle,N.J. (2002) Weight-cycling decreases incidence and increases latency of mammary tumor development to a greater extent than does chronic restriction in mouse mammary tumor virus-transforming growth factor-α female mice. *Cancer Epidemiol.Biomarkers Prev.*, **11**, 836-843.

7. Pape-Ansorge,K.A., Grande,J.P., Christensen,T.A., Maihle,N.J., and Cleary,M.P. (2002) Effect of moderate caloric restriction and/or weight-cycling on mammary tumor incidence and latency in MMTV-neu female mice. *Nutr.Cancer*, **44**, 161-168.

Attachment 1

REVISED STATEMENT OF WORK PC020457 2/13/03

Eliminating Specific Aim 5 primarily affects Original Task 6. If there is the opportunity to explore tissue analyses as indicated below it will be focused on aspects of IGF metabolism.

TASK 1 & 2. Establish breeding colony & set up genotyping assay.
MONTH 0-3. Order 6 male TRAMP mice (this is the maximum number that can be ordered at one time) and 25 nontransgenic female mice for breeding. Set up breeding. Set up genotyping assay and genotype mice produced. Rebreed mice to expand breeding colony.

TASK 3. Breed mice for EXPERIMENT 1A- LONGITUDINAL STUDY.
MONTHS 4-6. Breed mice to produce one third to one half of mice needed for this study. If one estimates 8 pups per litter, 1 out of 4 pups will be TRAMP males = two TRAMP males per litter. We will need a total of 160 TRAMP males = 80 litters. Genotype offspring. Assign mice to experimental groups. Set up immunohistochemistry assays.

TASK 4. Complete enrollment of mice for EXPERIMENT 1A and 1B SERIAL STUDY
MONTHS 7-12. Continue breeding to complete EXPERIMENT 1A. Three to four rounds of breeding will probably be needed to supply enough mice. Genotype mice as they are produced. Assign mice to experimental groups. Once longitudinal study is complete begin assigning mice to serial study for EXPERIMENT 1B.

TASK 5. Follow mice in EXPERIMENT 1A and 1B.
MONTHS 6-21. Monitor food intake, body weight and prostate tumor development in TRAMP mice. When age, tumor size and/or animal condition dictates euthanize mice and perform autopsies. Euthanize nontransgenic age-matched mice to correspond to those TRAMP mice with tumors. Euthanize mice that reach terminal ages of 48 or 50 wk of age. Record results and when study complete do statistical analyses of results.

ORIGINAL-TASK 6. Oncogene and tumor suppressor assays.
MONTHS 6-21. Order supplies and set up assays to perform p53, ErbB2 and possibly other growth factors for determination of gene expression and protein levels. Complete setting up assays and analyze samples as they become available.

REVISED-TASK 6. IGF-BP and IGF-I receptors. *Since we are focusing on IGF metabolism any work relating to gene expression will concentrate on factors related to IGF-I action such as IGF-I, IGF-I receptors and IGF-BP's.*

TASK 7. Restock breeding colony.
MONTHS 12-14. Evaluate breeding colony status and initiate breeding for EXPERIMENT 2-FASTING/REFEEDING study.

TASK 8. Enroll mice in FASTING/REFEEDING STUDY.
MONTHS 14-21. Breed mice, genotype offspring and enroll mice in FASTING/REFEEDING study. For this study 80-120 mice will be needed depended upon adding a PAIR-FED or a RESTRAINED group. We will have to follow the eating pattern of the FASTING/REFEEDING group for several months to determine if the additional group is needed.

TASK 9. Serum and tissue analyses and data analyses.

MONTHS 21-24 Complete tissue assays and when all animals are euthanized perform serum analyses and then complete data analyses of EXPERIMENT 1A and 1B.

TASK 10. Manuscript preparation for EXPERIMENT 1A and B.

MONTHS 25-26 Complete manuscript for the first experiment

TASK 11. Follow mice in FASTING/REFEEDING STUDY.

MONTHS 16-30. Monitor food intake, body weight and prostate tumor development in TRAMP mice. When tumor size dictates kill mice and perform autopsies. Kill nontransgenic mice to be age-matched to mice with tumors. Kill mice upon reaching 50 wk of age if still alive.

TASK 12. Analysis of tissue samples from FASTING/REFEEDING STUDY.

MONTHS 20-32. Perform assays on tumor and normal tissues from FASTING/REFEEDING STUDY as they become available.

TASK 13. Serum and tissue analyses of FASTING/REFEEDING STUDY.

MONTHS 28-32. Determine serum analyses from FASTING/REFEEDING as study groups are completed. Complete tissue analysis.

TASK 14. Compete statistical analysis of data from FASTING/REFEEDING STUDY.

MONTHS 33-34. Complete statistical analysis of data obtained from the FASTING/REFEEDING STUDY.

TASK 15. Prepare manuscript from FASTING/REFEEDING STUDY.

MONTHS 35-36. Write manuscript from results obtained from FASTING/REFEEDING STUDY.